Carotenoids, Cancer, and Clinical Trials

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In the early 1980s β-carotene was proposed to reduce the risk of cancer. Epidemiologic support for this hypothesis has come from three types of studies: prospective studies of dietary intake and cancer, prospective studies of blood β-carotene levels and cancer, and retrospective studies of dietary intake and cancer.[1,2] In the prospective studies, dietary information and/or blood samples are collected from a group of nondiseased people; and the cohort is followed over time. When a sufficient number of cancer diagnoses or deaths have occurred, the data collected earlier are compared for the cases and either all the noncases in the cohort or a subset of the cohort matched to the cancer cases. In a retrospective study patients with a particular cancer are identified, and comparable control subjects selected. Then information about usual diet prior to symptoms of disease is collected and compared for the cases and controls.

An example of a prospective study involving dietary assessment is the 19-year follow-up of approximately 2000 middle-aged Western Electric Company employees living in Chicago.[3] In the 33 men that subsequently developed lung cancer, provitamin A carotenoids were the only nutrient of those evaluated significantly associated with reduced risk (TABLE 1). The risk of lung cancer was seven times higher in men in the lowest quartile of carotenoid intake than in men in the highest quartile. Other common cancers were not significantly associated with intake of provitamin A carotenoids although carotenoid intake was lower than average in men who subsequently developed head and neck cancers.

An example of a prospective study involving direct measurement of β-carotene in blood is the 10-year follow-up of approximately 7000 Hawaiian Japanese men participating in the Honolulu Heart Program.[4] In a case-cohort analysis of 74 incident lung cancers, age- and smoking-adjusted relative risks of lung cancer increased with serum β-carotene levels; the test for trend was statistically significant (TABLE 2). Men in the lowest quintile of serum β-carotene levels had twice the risk of men in the highest quintile. No significant associations were noted for colon, stomach, rectal, or bladder cancer although β-carotene levels were somewhat lower in the men who subsequently developed colon and stomach cancers than in the controls. There was no association of either serum vitamin A or serum vitamin E with any of the cancers.

Lung has been the cancer site most frequently studied in retrospective studies of carotenoid intake and cancer. In one of the earliest studies to distinguish between carotenoid and vitamin A intake, a population-based case-control study of lung cancer in white men in six high-risk areas of New Jersey,[5] men in the lowest quartile of carotenoid intake had a smoking-adjusted relative risk of 1.7,

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TABLE 1. Dietary Intake in Men Who Subsequently Developed Lung Cancer and Those Who Did Not During 19 Years of Follow-up of Western Electric Workers in Chicago[a]

Nutrient	*p* for Difference between Means*
Carotene index	<0.001
Retinol index	0.38
Energy intake	0.27
Animal protein (%cal)	0.93
Vegetable protein (%cal)	0.10
Animal fat	0.84
Vegetable fat	0.78
Carbohydrate	0.07
Calcium	0.11
Phosphorous	0.26
Iron	0.18
Thiamin	0.19
Riboflavin	0.27
Niacin	0.10
Vitmain C	0.20
Vitamin D	0.85
Cholesterol	0.59

* Based on Student *t* test.
[a] Derived from Shekelle *et al.*[3]

relative to men in the highest quartile; but no increase in risk was associated with low retinol (preformed vitamin A) intake (TABLE 3). The protective effect was limited to current and recent smokers. Intakes of vegetables, dark green vegetables, and dark yellow-orange vegetables showed stronger associations than the carotenoid index. The smoking-adjusted relative risks of those in the lowest quartiles of consumption of these food groups were 1.8–2.2, compared to those in the highest quartiles. Two explanations were proposed. Dark green and dark yellow-orange vegetable consumption might be better measures of β-carotene intake than an approximate estimate of provitamin A carotenoids. Alternatively, there might be protective entities in vegetables and fruits in addition to the carotenoids.

TABLE 2. Relative Risks of Lung Cancer in Hawaiian Japanese Men by Quintiles of Serum β-Carotene Concentration[a]

			Relative Risks	
	Cases	Controls	Unadjusted	Adjusted for Age, Smoking
β-Carotene concentration (μg/dl)				
57.1–311.5	7	60	1.0	1.0
34.6–57.0	12	60	1.7	1.5
25.1–34.5	10	59	1.5	1.2
15.1–25.0	21	62	2.9	2.4
0–15.0	24	61	3.4	2.2
p for trend			0.004	0.04

[a] Derived from Nomura *et al.*[4]

TABLE 3. Smoking-Adjusted Relative Risks of Lung Cancer by Nutrient and Food Group Intake in New Jersey White Male Current and Recent Cigarette Smokers[a,b]

Nutrient or Food Group	Level of Consumption			p for Trend
	Upper 25%	Middle 50%	Lower 25%	
Retinol	1.0	1.1	1.0	0.48
Carotenoids	1.0	1.5	1.7	0.02
Vitamin A	1.0	1.2	1.2	0.26
Dairy products	1.0	0.8	0.9	0.26
Vegetables and fruit	1.0	1.7	1.8	0.005
Fruit	1.0	1.4	1.2	0.28
Vegetables	1.0	1.3	1.7	0.004
Dark green vegetables	1.0	1.4	1.8	0.002
Yellow-orange vegetables	1.0	1.6	2.2	<0.001

[a] Included are 524 cases and 354 controls.
[b] Derived from Ziegler *et al.*[5]

In these and many other epidemiologic studies, low intake of carotenoids, vegetables, and fruits is consistently associated with increased risk of lung cancer—in both prospective and retrospective studies. Low levels of β-carotene in serum or plasma are consistently associated with the subsequent development of lung cancer. The simplest explanation is that β-carotene is protective. Since retinol is not related in a similar manner to lung cancer risk, β-carotene appears to function through a mechanism that does not require its conversion into vitamin A. Prospective and retrospective studies also suggest that vegetable and fruit intake may reduce the risk of many other cancers; specifically, cancers of the digestive (mouth, pharynx, esophagus, stomach, pancreas, colon, rectum), respiratory (larynx), and urinary tracts (bladder). Whether vegetable and fruit intake influences hormone-related cancers is unclear. Weaker protective effects have been noted for breast cancer than for many other cancers and are not consistently seen. Pertinent data for ovarian and endometrial cancer are limited. Results for prostate cancer are not consistent. Little relevant research exists for a number of cancer sites, such as the lymphatic and hematopoietic cancers. In addition, evidence of reduced risk with high vegetable and fruit intake does not necessarily imply that the dietary etiology is the same as that observed with lung cancer. For example, β-carotene does not seem to explain the reduced risk of esophageal and stomach cancer associated with increased vegetable and fruit intake.

In a population-based case-control study of esophageal cancer in black men in Washington, DC,[6] low vegetable and fruit consumption was significantly associated with an increased risk of esophageal cancer, but so also were low dairy product and egg consumption and low fresh or frozen meat and fish consumption (TABLE 4). Low intake of vitamin C, riboflavin, and vitamin A, as well as of carotenoids, were all associated with elevated risk. These results suggested that generally poor nutrition, characterized by inadequate intake of the basic food groups, was the dominant dietary risk factor. Multiple micronutrient deficiencies might be involved. This explanation is consistent with the geographic pattern of this cancer. Internationally it is endemic in regions with limited diets and

TABLE 4. Ethanol-Adjusted Relative Risks of Esophageal Cancer by Food Group and Nutrient Intake in Washington, DC Black Males[a,b]

Nutrient or Food Group	Level of Consumption		
	Upper 33%	Middle 33%	Lower 33%
Meat and fish	1.0	1.3	0.9
Dairy and eggs	1.0	1.6	2.0^{++}
Vegetables and fruits	1.0	2.1	2.4^{+++}
Vegetables	1.0	1.7	1.8^{++}
Green vegetables	1.0	1.2	1.5^{+}
Yellow vegetables	1.0	1.0	1.2
Fruits	1.0	2.8	2.4^{+++}
Carbohydrates	1.0	1.1	1.2
Fresh or frozen meat and fish	1.0	1.5	2.1^{++}
Precooked or processed meat and fish	1.0	0.9	0.8
Vitamin A	1.0	1.4	1.5
Carotenoids	1.0	1.4	1.6^{+}
Vitamin C	1.0	1.3	2.1^{+++}
Thiamin	1.0	1.2	1.1
Riboflavin	1.0	1.1	1.6^{+}

[a] Included are 120 cases and 250 controls. Statistical significance of trends: $^{+}$ $p < 0.10$, $^{++}$ $p < 0.05$, $^{+++}$ $p < 0.01$.
[b] Derived from Ziegler *et al.*[6]

impoverished agriculture; within a country it is associated with low socioeconomic status.

In a population-based case-control study of stomach cancer conducted in high- and low-risk areas of Italy,[7] intake of not only β-carotene but also of vitamin C were inversely associated with risk (TABLE 5). However, the associations with β-carotene were weakened in multivariate analyses adjusting for other nutrients. A number of other epidemiologic studies have demonstrated an increased risk of stomach cancer with decreased intake of vitamin C, and it is frequently postulated that vitamin C inhibits the endogenous formation of potentially carcinogenic N-nitroso compounds by preventing the nitrosation of secondary and tertiary amines. Nonetheless in this study, raw vegetables, citrus fruit, and other fresh fruit were at least as protective as vitamin C, suggesting that other constituents in vegetables and fruits might also be important.

Numerous epidemiologic studies, from 75 to 200 depending on the criteria, have demonstrated associations between increased intake of vegetables and fruits and reduced risk of cancer at many, though not all, sites.[1,2,10–12] Indeed many epidemiologists believe that of all the dietary factors postulated to be related to cancer, including fat and calories, the epidemiologic evidence is the most consistent for vegetables and fruits. The public health implications are impressive. In many studies cancer risk among individuals in the highest one-fifth to one-third of vegetable and fruit intake is 50–80% the risk among those in the lowest one-fifth to one-third of intake. This association, if causal, when translated into attributable risk,[13] implies that 10–33% of potential cancers would be prevented were all in the population to adopt the levels of vegetable and fruit intake characteristic of the highest quantile. The lowest two-thirds to four-fifths of the population, lowest in terms of vegetable and fruit intake, stand to benefit. Multiple opportunities for

change can be envisioned: first and foremost, increasing vegetable and fruit intake, and once the etiologic important constituents of vegetables and fruits are identified, incorporating them into dietary supplements, fortified foods, and genetically engineered crops.

However, there are alternative explanations for the reduction in cancer risk associated with intake of carotenoids, vegetables, and fruits. β-carotene may be the simplest, but not the only, answer. First, avoidance of smoking, limited drinking, regular physical activity, and judicious utilization of medical care often accompany increased vegetable and fruit intake, and probably explain part of the apparent reduction in risk. Second, other beneficial dietary patterns, such as reduced intake of fat and calories and reduced percent of calories from fat, may be related to increased vegetable and fruit consumption. Third, nutrients other than β-carotene that are concentrated in vegetables and fruits, such as vitamin C and dietary fiber; other carotenoids; and constituents of vegetables and fruits that are not nutrients may play important roles.

To evaluate the possibility that correlated dietary patterns explain the impact of vegetable and fruit intake, we used two nationally representative dietary surveys to identify the nutrients, food groups, and food preparation practices strongly correlated with high vegetable and fruit intake. We used the 115-item food frequency interview administered to 10,000 adults in the 1982–84 Epidemiologic Follow-up (EFS) of the First National Health and Nutrition Examination Study (NHANES I)[9] and the 60-item food frequency interview administered to 20,000 adults in the 1987 National Health Interview Survey.[14] Results from the two data sets were similar. Percentage of calories from fat was inversely correlated with vegetable and fruit intake ($r = -0.25$), although absolute intake of saturated fatty acids, polyunsaturated fatty acids, and cholesterol seemed unrelated. However, since the correlation with percent of calories from fat was only moderate, its

TABLE 5. Adjusted Relative Risks of Stomach Cancer by Food Group and Nutrient Intake in Low- and High-Risk Areas of Italy[a,b]

	Level of Consumption			
	Lower 33%	Middle 33%	Upper 33%	*p* for Trend
Food groups				
Raw vegetables	1.0	0.8	0.6	<0.001
Cooked vegetables	1.0	0.9	1.1	0.58
Beans	1.0	0.8	0.8	0.10
Onions and garlic	1.0	1.0	0.8	0.04
Citrus fruit	1.0	0.7	0.6	<0.001
Other fresh fruit	1.0	0.6	0.4	<0.001
Dried and preserved fruit	1.0	0.8	1.0	0.87
Bread and pasta	1.0	1.1	1.0	0.99
Milk and dairy products	1.0	1.0	1.1	0.49
	Lower 20%	Middle 20%	Upper 20%	
Nutrients				
Vitamin C	1.0	0.6	0.5	
β-carotene	1.0	0.6	0.6	

[a] Included are 1016 cases and 1159 controls.
[b] Derived from Buiatti *et al.*[7,8]

TABLE 6. Categorization of 1982–84 NHANES I EFS Participants by Vegetable and Fruit Intake and Percent of Calories from Fat

Percent Calories from Fat	Vegetables and Fruits: Lowest Quartile	Quartile 2	Quartile 3	Highest Quartile
Highest quartile	9.8%	7.3%	5.1%	2.9%
Quartile 3	6.2%	7.0%	6.4%	5.4%
Quartile 2	4.8%	6.0%	6.8%	7.4%
Lowest quartile	4.3%	4.7%	6.7%	9.3%

effects should be separable from that of vegetables and fruits in a sufficiently large epidemiologic study. As demonstrated in TABLE 6, only 33% of the population was ranked in the equivalent quartiles for both exposures (Q1/Q1 + Q2/Q2 + Q3/Q3 + Q4/Q4; 25% would be expected if the exposures were statistically independent); and almost as many, 27%, differed by more than one quartile (Q1/Q3 + Q1/Q4 + Q2/Q4 + Q3/Q1 + Q4/Q1 + Q4/Q2).

A second line of research focuses on whether β-carotene is uniquely protective, or whether high β-carotene blood levels may simply be an indicator of increased intake of all carotenoids and of vegetables and fruits in general. As we began to evaluate liquid chromatography (LC) methods for separating and quantifying the major individual carotenoids in human serum and plasma, it became apparent that there was little published information on recovery of individual and total carotenoids. One reason was the lack of availability of pure reference materials for carotenoids other than β-carotene. Another was that cancer research was narrowly focused on β-carotene. Poor recovery during measurement of a carotenoid can lead to an imprecise estimate of exposure and thus obscure an association. In addition, spurious associations can be generated by differential recovery between cases and controls.

In collaboration with the National Institute of Standards and Technolgoy, we decided to develop a LC method for measuring individual carotenoids in human serum and plasma that would optimize resolution and recovery. In addition, the method had to be reproducible and practical so that it could be used by a variety of laboratories on the large numbers of samples collected in epidemiologic studies. Percentage recoveries of the common serum carotenoids (lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene) with the NIST-NCI method[15,16] and with three accepted LC methods that have been used in epidemiologic studies are presented in TABLE 7. Recovery was measured by flow injection analysis. The NIST-NCI method gives 93–99% recovery of each of the six carot-

TABLE 7. Percent Recovery of Individual Carotenoids with Different LC Methods

Method	Lutein[a]	Zeaxanthin[a]	β-Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
NIST-NCI	95	94	93	97	99	99
A	80	75	82	68	89	91
B	99	98	85	70	77	84
C	99	91	96	101	94	91

[a] Lutein and zeaxanthin coelute in all methods except NIST-NCI.

enoids. Recovery drops to 70% or less for lycopene and to 80% or less for at least one additional carotenoid with methods A and B. Method C gives quite good recovery of all six carotenoids, but its resolution of individual carotenoids is limited. Of the four methods, only the NIST-NCI method can resolve the structural isomers lutein and zeaxanthin.

The ability of the NIST-NCI method to separate individual serum carotenoids is demonstrated in FIGURE 1. Not only are structural isomers that frequently coelute (α-cryptoxanthin and β-cryptoxanthin, and lutein and zeaxanthin) resolved but geometric isomers are also separated. The two small peaks trailing the all-

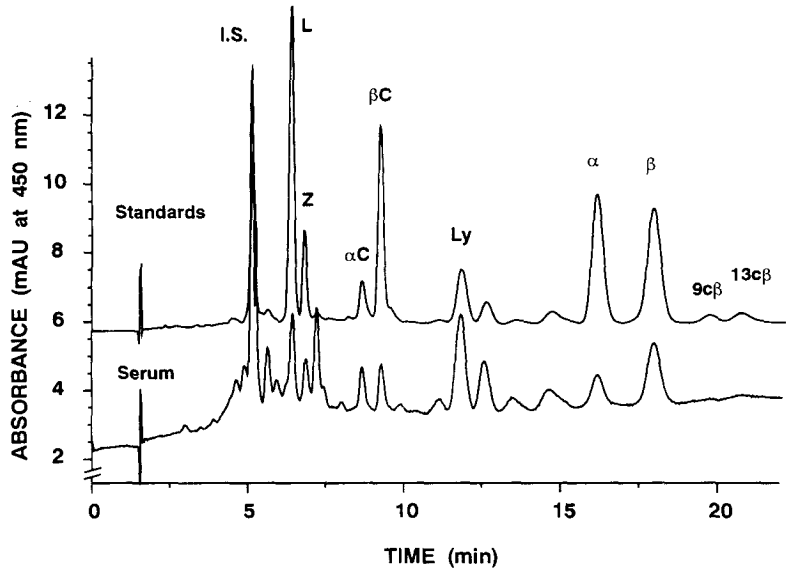

FIGURE 1. Resolution of serum carotenoids with the NIST-NCI LC method. *Upper tracing*: mixture of the six most common serum carotenoids: lutein (L), zeaxanthin (Z), β-cryptoxanthin (βC), lycopene (Ly), α-carotene (α) and β-carotene (β); also seen are α-cryptoxanthin (αC), several unlabeled geometric isomers of lycopene, 9-cis-β-carotene (9cβ), and 13-cis-β-carotene (13cβ). *Lower tracing*: extract of human serum. The internal standard (I.S.) is β-apo-8'-carotenal.

trans β-carotene peak are the 9-*cis* and 13-*cis* isomers, which together comprise approximately 10% of total serum β-carotene. The two peaks after all-*trans* lycopene and the peak before it contain its geometric isomers.

Several projects are now underway that utilize the NIST-NCI method for measuring individual carotenoids. With stored serum from the Japanese men in the Honolulu Heart Program cohort,[4] we are investigating the relationships of individual and total carotenoids in serum to subsequent incidence of lung, oral, pharyngeal, and esophageal cancer. With serum collected from community controls in a large case-control study of cervical cancer,[17] we are analyzing the distribution and demographic, socioeconomic, and lifestyle determinants of indi-

vidual and total carotenoids in five areas of the United States. Finally, we are comparing in the laboratory the resolution, recovery, and reproducibility of the LC methods commonly used in epidemiologic studies of carotenoids, and quantitatively evaluating how the methods limit epidemiologic analysis and interpretation.

Other carotenoids are not the only constituents of vegetables and fruits that merit further investigation from the perspective of cancer etiology. Vitamin C, like β-carotene found primarily in vegetables and fruits, is a promising candidate.[18] Vegetables and fruits also contribute to the intake of dietary fiber, folate, and vitamin E, each of which may be protective. Other compounds in vegetables and fruits which are not nutrients, such as the dithiolthiones, flavonoids, glucosinolates and indoles, isothiocyanates, phenols, phytoestrogens, sterols, protease inhibitors, and allium compounds, may be important.[11] Although these compounds may not be essential for health or growth, they may play a role in reducing the risk of chronic disease. It is quite likely that at least several protective factors exist in vegetables and fruits and that mechanisms differ for the various cancers. Because the protective factors have not yet been conclusively identified, NCI currently advocates, through its "Five A Day for Better Health" program, consuming at least five servings of vegetables and fruits a day.

Chemoprevention trials will soon begin to answer to what extent β-carotene can explain the reduction in cancer risk consistently associated with increased vegetable and fruit consumption. Data from two of these trials, conducted in Linxian, China[19,20] and in Finland,[21] are now being analyzed, and will be published in 1993 or 1994. Linxian was selected as the site for two randomized placebo-controlled intervention trials of multiple vitamin and mineral supplementation because of its extraordinarily high rates of esophageal and gastric cancer, both of which are associated with low vegetable and fruit intake. Food availability and variety in Linxian have historically been limited, and subclinical deficiencies of several micronutrients have been demonstrated. In the dysplasia trial 3318 subjects, aged 40–69, with cytologically diagnosed dysplasia of the upper gastrointestinal tract were assigned to receive a daily supplement containing 14 vitamins, 12 minerals, and 15 mg of β-carotene or a placebo.[19] Doses were typically 2–3 times the U.S. Recommended Daily Allowances. During the six years of the trial, 462 cancers accrued: 262 of the esophagus and 179 of the stomach, primarily in the cardia region. In the general population trial, 29,584 volunteers, aged 40–69, were randomly assigned to intervention groups according to a one-half replicate of a 2^4 factorial experimental design.[20] The design enabled testing for the effects of four combinations of nutrients: retinol and zinc; riboflavin and niacin; vitamin C and molybdenum; and β-carotene (15 mg), α-tocopherol, and selenium. Micronutrient levels were typically at 1–2 times the U.S. Recommended Daily Allowances. In the 5.25 years of the trial 1307 cancers accrued: 639 esophageal and 546 gastric, primarily in the cardia region.

In the U.S.-Finland lung cancer prevention trial, as in the Linxian trials, a high-risk population in an area of low vegetable and fruit consumption is being investigated. In the past decade Finland has experienced lung cancer incidence rates among the highest in the world, due mainly to the high proportion of smoking males. In a randomized, placebo-controlled trial 29,246 males in Finland, aged 50–69, who smoked 5 or more cigarettes a day were assigned to receive a daily supplement of 20 mg of β-carotene, 50 mg of α-tocopherol, both micronutrients, or neither.[21] During the 5–8 years of the trial approximately 800 cases of lung cancer have accrued, as well as sufficient numbers of prostate, colorectal, and bladder cancer for analysis.

If definitive or marginal protection for one or several cancers is demonstrated

in any of these chemoprevention trials, then the results will provide direction for further research and for public health policy. However, negative trials with no evidence of reduced cancer risk will not conclusively rule out a role for micronutrients. The micronutrient levels might have been too low; pharmacologic dosages might have been necessary. Supplements might have been taken for too short a period of time, or too late for a crucial period in carcinogenesis. Other cancer sites or other populations with different exposures might have been more responsive. Other combinations of micronutrients or broader changes in diet might have been more effective. Nonetheless, the results of these early micronutrient supplementation trials will hopefully contribute significantly to our understanding of the role of β-carotene in cancer etiology.

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Carotenoids, Cigarette Smoking, and Mortality Risk[a]

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INTRODUCTION

Epidemiological studies have repeatedly shown that cancer risk is lower among individuals with high intake of foods that contain carotenoids or with high blood levels of one particular carotenoid, beta-carotene.[1,2] There is also evidence, although considerably less extensive and conclusive, that beta-carotene may lower the risk of cardiovascular disease.[3,4] In industrialized countries cancer and cardiovascular disease together account for roughly two thirds of all deaths, and a strong protective effect of carotenoids should therefore translate into a lower risk of overall mortality. Nevertheless, virtually all of the studies published to date have focused on specific diseases rather than total mortality associated with beta-carotene or other carotenoids. Also, the health benefits observed in epidemiological studies among people who ingest more carotenoids and who have higher blood levels could be due to other components in their diets[5] or to nondietary factors. The most important concern in this regard is cigarette smoking, since smokers on average consume fewer fruits and vegetables than nonsmokers,[6,7] and their blood levels of carotenoids are also lower.[8,9] To examine further the possible relationship between mortality risk, beta-carotene intake, and smoking we have continued to follow patients enrolled in a clinical trial of beta-carotene to prevent nonmelanoma skin cancer. We present here the preliminary results of our posttreatment follow-up survey, for data through 1 March 1991.

METHODS

The design and principal results of the Skin Cancer Prevention Study were reported previously.[10,11] Briefly, this was a randomized, double-blind clinical trial

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